Microbial hydrocarbon gases in the Witwatersrand Basin, South Africa: Implications for the deep biosphere


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Abstract—In this study, compositions and $\delta^{13}C$ and $\delta^2H$ isotopic values of hydrocarbon gases from 5 mines in the Witwatersrand basin, South Africa, support the widespread occurrence of microbiologically produced methane in millions of years-old fissure waters. The presence of microbial methane is, to a large extent, controlled by the geologic formations in which the gases are found. Samples from the Witwatersrand Supergroup have the largest microbial component based on $\delta^{13}C$ and $\delta^2H$ signatures and CH$_4$/C$_2+$ values. Based on mixing between a microbial CH$_4$ component and a more $^{13}C$-enriched and $^2H$-depleted C$_2+$-rich end member, conservative estimates of the % contribution of microbial CH$_4$ to the gas samples range from $>90\%$ microbial CH$_4$ at Beatrix, Masimong, and Merriespruit, to between 5 and 80% microbial CH$_4$ at Evander, and <18% microbial CH$_4$ at Kloof. The Witwatersrand basin’s history of thermal alteration of organic-rich ancient sedimentary units suggests a thermogenic origin for this $^{13}C$-enriched end member. Alternatively, the potential for an abiogenic origin similar to hydrocarbon gases produced by water-rock interaction at other Precambrian Shield sites is discussed. Microbial methane is predominantly found in paleo-meteoric fissure waters with $\delta^18O$ and $\delta^2H$ values that fall on the meteoric waterline, and have temperatures between 30 to 40°C. In contrast, fissure waters with a larger component of nonmicrobial hydrocarbon gases show a trend towards more enriched $\delta^{18}O$ and $\delta^2H$ values that fall well above the meteoric waterline, and temperatures of 45 to 60°C. The enrichment in $^{18}O$ and $^2H$ in these samples, and their high salinity, are similar to the isotopic and compositional characteristics of saline groundwaters and brines produced by water-rock interaction at Precambrian Shield sites elsewhere. The reported 100 Ma ages of fissure waters from the Witwatersrand and Ventersdorp formations suggest that these microbial hydrocarbon gases are the product of in situ methanogenic communities in the deep subsurface of the Witwatersand basin. Small subunit ribosomal RNA genes were amplified using archaeal-specific primer sets from DNA extracts derived from several of these waters. Fissure waters with a high proportion of microbial methane also contained sequences resembling those of known methanogens.

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1. INTRODUCTION

Substantial quantities of hydrocarbon gases have been observed within Precambrian rocks of the Canadian Shield (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b), the Fennoscandian Shield (Nurmi et al., 1988; Sherwood Lollar et al., 1993a), the Khibina, Lovozero, and Kovdor intrusions of the Kola Alkaline Province in Russia (Nivin et al., 1995; Potter et al., 1998; Voytov, 1991), and the Witwatersrand basin in South Africa (Cook, 1998). In general, hydrocarbon gases can be divided into two types based on origin. Biogenic hydrocarbon gas refers to gas produced directly by microorganisms during respiratory and fermentative processes (microbial gas); or, it can refer to methane and higher hydrocarbons derived from the thermal breakdown of more complex organic matter (thermogenic gas) (Schoell, 1988). Biogenic processes are the most common means of hydrocarbon gas formation, and a substantial body of literature and experience has been established to characterize these sources based on isotopic and compositional analyses (Schoell, 1983; Schoell, 1984; Whiticar et al., 1986; Schoell, 1988; Hunt, 1996). At a number of Precambrian Shield sites, the presence of abiogenic hydrocarbon gases has been suggested (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b; Nivin et al., 1995; Sherwood Lollar et al., 2002b; Potter and Konnerup-Madsen, 2003). Abiogenic synthesis of hydrocarbons involves the production of gases without the action of microorganisms or the breakdown of a high molecular weight organic precursor (Schoell, 1988). Mantle derived abiogenic gases have a CH$_4$ component that is significantly enriched in $^{13}C$ compared to microbial or thermogenic CH$_4$ due to the juvenile carbon source (Welhan and Craig, 1979; Welhan, 1988; Abrajano et al., 1888). Abiogenic hydrocarbon gases can also be produced from crustal carbon sources (graphite, CO, CO$_2$, or dissolved inorganic carbon) by processes such as surface-catalysed polymerization (Anderson, 1984); metamorphism of graphite-carbonate bearing rocks (Giardini and Salotti, 1969; Holloway, 1984; Kenney et al., 2002); and other gas-water-rock alteration reactions in...
the presence of catalytically active metals, such as serpentinization (Vanko and Stakes, 1991; Charlou and Donval, 1993; Berndt et al., 1996; McCollom and Seewald, 2001; Kelley et al., 2001). Experimental results confirm that production of abiogenic CH$_4$ from crustal carbon sources by water-rock interactions can result in $\delta^{13}C$ values as depleted as $-50\%$ (Yuen et al., 1990; Hu et al., 1998; Horita and Berndt, 1999).

Within the hard rock mines of Witwatersrand basin, South Africa, hydrocarbon gases, saline groundwaters, and brines are encountered when drilling into faults and/or dyke structures (Cook, 1998). Some compositional analyses of the subsurface gases have been obtained by various mines (Cook, 1998). Few analyses, however, have been done to determine both compositions and stable isotopic signatures, which are the most reliable means of identifying the origin of hydrocarbon gases. It was first suggested that the gases within the Witwatersrand basin originated from shallow coal deposits in the basin and were transported as dissolved phases in groundwater into the underlying strata via cross-cutting igneous dykes intruded into the Witwatersand sediments (Eschenburg, 1980; Tweedie, 1986; Robb and Meyer, 1995; Cook, 1998). Others have suggested that the gases were formed within the basin by the thermal conversion of shale layers and/or thin carbon seams (Cook, 1998). Alternatively, the gases could be similar to abiogenic gases found in other Precambrian Shield environments such as the Canadian and Fennoscandian Shields (Sherwood Lollar et al., 1993a; Sherwood Lollar et al., 1993b; Sherwood Lollar et al., 2002b).

A growing body of evidence supports the presence of indigenous microbial communities to considerable depth in the Witwatersrand basin, and thus, the possibility that at least some of the methane is microbially produced must be considered. Known microbes capable of producing methane gas are confined to only one domain of life, the Archaea (Woese and Olsen, 1986). Anaerobic microorganisms of various physiotypes have been cultivated from boreholes receiving Witwatersrand basin fissure waters (Kiefert et al., 1999; Takai et al., 2001). However, to date, the report of Bonin and Boone (2004) represents the only documented cultivation of methanogens. Detailed examinations of fissure water microbial community structure have been conducted using culture-independent approaches (e.g., small subunit ribosomal RNA gene sequences (SSU rDNA) (Takai et al., 2001; Baker et al., 2003; Moser et al., 2003a; Moser et al., 2003b), or phospholipid fatty acid (Pflüffer et al., 2000)). Archaeal SSU rDNA sequences resembling those of methanogens have been detected in a previous survey of Witwatersrand deep mine samples, but were limited to mine service water and dolomite aquifer samples (Takai et al., 2001).

The objective of this study was to use gas compositions and stable isotope signatures to constrain the origin of hydrocarbon gases at five sites located in the Witwatersrand and Ventersdorp formations in the Witwatersrand basin, South Africa. These data were coupled with archaeal SSU rDNA sequences amplified by polymerase chain reaction (PCR) from microbial cells filtered from associated fracture waters from a number of the boreholes for which gases were sampled. The presence or absence of sequences resembling those of known methanogenic archaebacteria was employed as a qualitative screen for methanogens in these samples. Here we report on patterns of microbially produced hydrocarbons (or mixtures thereof) that correspond with the host rock type and support this geochemical and isotopic evidence with rDNA sequences for likely methanogens.

2. GEOLOGICAL SETTINGS AND SAMPLING LOCATIONS

The Witwatersrand basin is a large Archean intracratonic basin composed of volcanosedimentary sequences divided chronologically into the schist basement, the sedimentary quartzite and shale layers of the Witwatersrand Supergroup, and the andesitic lava sequence of the Ventersdorp Supergroup (Coward et al., 1995). The NE-SW elongated basin (Fig. 1) is approximately 360 km by 200 km. Evander Mine and Middelbult Colliery are located in the northeast portion of the basin. The Witwatersrand Supergroup (approx. 3000 to 2800 Ma) overlies the schist basement (>3070 Ma) and is the main focus for mining in the Witwatersrand basin (Fig. 2a). The Witwatersrand Supergroup has been metamorphosed to lower green-schist facies and divided into two groups based on depositional characteristics. The lower part of the Witwatersrand Supergroup (the West Rand Group) is composed of marine distal shelf facies with a minor intertidal component. They are characterized as sands, greywacke, shales, and argillites with minor-banded ironstones and can reach a maximum thickness of 7500m (Coward et al., 1995). The upper part of the Witwatersrand Supergroup (Central Rand Group) is composed of fragments derived from the eroded basement. It is characterized by an upwards-coarsening depositional pattern of fluvial sands, quartzites and conglomerates with minor shale layers that reach a maximum thickness of 2900m. Overlying the Central Rand Group is the thick sequence of andesitic lavas that comprises the Ventersdorp Supergroup (2700 Ma). This layer is comprised of up to 1840m of bimodal volcanics, thinner layers of sandstone and conglomerate, and a layer of tholeitic flood basalt (Coward et al., 1995). The organic-rich Black Reef quartzite deposit marks the transition between the Ventersdorp Supergroup and the Transvaal Supergroup (2000–2500 Ma) (Tweedie, 1986; Coward et al., 1995). The Transvaal sediments consist of a thick dolomitic unit overlain by terrigenous sediments. Uplift of the Vredefort Dome at the centre of the Witwatersrand basin (2025 Ma) (Fig. 1) resulted in deformation of the Transvaal sediments, the Ventersdorp, and the Witwatersrand Supergroups (Coward et al., 1995). Eroded, uplifted structures gave rise to the younger (~200 million yr old) Karoo sedimentary basin. The Karoo sequence is composed mainly of interbedded shale and sandstone layers only a few hundred meters in thickness, with a thin layer (5m) of low-grade bituminous coal near its base (Tweedie, 1986). At Evander, samples were collected from boreholes in the underground workings in the Witwatersrand Supergroup at depths between 1474 mbls (meters below land surface) and 1950 mbls (Fig. 2a). In this area of the basin, the Transvaal has been partially removed by pre-Karoo erosion. At Middelbult, samples were collected from boreholes extending from surface to 122 to 200 mbls to the bituminous coal seam at the base of the Karoo sediments. Kloof Mine is located in the northwest section of the Witwatersrand basin where the Karoo sediments have been removed by erosion (Fig. 2b) (Johnson et al., 1996). Gas samples
at this site were exclusively from the Ventersdorp Supergroup at depths of 3300 to 3400 mbls. Beatrix, Merriespruit, and Masimong Mines are all located in the southern part of the basin near Welkom, where the Karoo sediments unconformably overlie the Ventersdorp Supergroup (Fig. 2c). All samples in these mines were collected from underground boreholes in the Central Rand Group of the Witwatersrand Supergroup. Samples at Beatrix were from 718 to 1390 mbls, while samples from Masimong and Merriespruit Mines were from 1880 and 2028 mbls respectively.

Carbonaceous matter occurs in the form of stratiform carbon seams and spherical solid bitumen nodules throughout the Witwatersrand basin (Spangenberg and Frimmel, 2001). The seams are only a few centimeters thick, and nodules of solid bitumen are up to 1 cm in diameter. They are typically found on bottom scour surfaces and wrapped around quartzite pebbles within the West Rand and Central Rand Groups (Carbon Leader/Ada May or Main Reef), within the Central Rand Group (Bird/Beatrix/Kimberly Reefs), at the base of the Ventersdorp Supergroup (Ventersdorp Contact Reef), and between the Ventersdorp Supergroup and the Transvaal sediments (Black Reef) (Figs. 2 a–c; Spangenberg and Frimmel, 2001). The source has been the subject of considerable debate (Robb and Meyer, 1995), but the general consensus is that it originated from late Archean algal-bacterial kerogen derived from extensive microbial mats that once covered the surface of the Witwatersrand elastic sediments (Spangenberg and Frimmel, 2001).

3. MATERIAL AND METHODS

All samples were collected at the borehole collar. A packer was placed into the opening of the borehole and sealed to the inner rock walls below water level to seal the borehole from the mine air and minimize air contamination. Gas and water were allowed to flow through the apparatus long enough to displace any air remaining in the borehole or the apparatus before sampling. Plastic tubing was attached to the end of the packer, and the flow of gas and/or water from the borehole was directed into a graduated sampling bucket. If both gas and water flowed from the borehole, both flows were measured. The water flow rate was determined from the average time required for each of three fillings of a graduated sampling bucket. Gas flow was measured by filling the sampling bucket with water, then displacing a known volume of water from an inverted graduated beaker after Fritz et al. (1987). When there was no water, but flowing gas present in the borehole, the gas flow was measured in an inverted cylinder from a bucket filled with mine service water (water used in mining and drilling operations). For each borehole, gas flow rates were averaged from three measurements. In several cases, gas and water flow rates varied significantly during sampling, so a range is reported (Table 1).

Following flow measurement, gases collected in the inverted beaker were transferred directly into preevacuated vials through a 22-g syringe needle on a luer attachment at the top of the beaker. The gas sampling
vials were preevacuated 130 ml borosilicate vials sealed with butyl blue rubber stoppers. All stoppers were pretreated by boiling in 0.1 mol/L NaOH for an hour (Oremland and Des Marais, 1983). Vials were prefixed with 50 μL of a saturated HgCl2 solution to kill any microbes contained in the sample so microbial activity postsampling would not alter the gas composition and isotopic signatures. Six vials were filled from the gas collected in the inverted beaker and overpressurized by adding 20cc of the borehole water. All samples were stored inverted at room temperature until analysis was performed within three months following sampling. This amount of storage time has been previously shown to have no effect on the isotope signatures of the gases (Ward, 2002).

Compositional analyses of gas samples were performed at the Stable Isotope Laboratory at the University of Toronto. A Varian 3400 gas chromatograph (GC) equipped with a flame ionization detector (FID) was used to determine the concentrations of CH4, C2H6, C3H8, and C4H10. The hydrocarbons were separated on a J&W Scientific GC-Q column (30 m x 0.32 mm ID) with a helium gas flow and the following temperature program: initial 60°C hold 2.5 min, increase to 120°C at 5°C/min. A Varian 3800 GC equipped with a micro-thermal conductivity detector (μTCD) and a Varian Molecular Sieve 5A PLOT fused silica column (25m x 0.33mm ID) were used to determine the concentrations of the inorganic gas components (H2, He, Ar, O2, CO2, and N2).

To determine the concentrations of Ar, O2, and N2, the helium carrier gas flow rate was 50ml/min and the temperature program was: initial 60°C, increase to 250°C at 20°C/min, hold 6 min. To determine the concentration of H2 and He, the argon carrier gas flow rate was 2 ml/min and temperature program was: initial 10°C hold 10 min, increase to 80°C at 25°C/min, hold 7 min. All analyses were run in triplicate and mean values are reported. Reproducibility for triplicate analyses was ±5%.

Stable carbon and hydrogen isotopic analysis for all hydrocarbons was performed at the Stable Isotope Laboratory at the University of Toronto. Analyses for δ13C values were performed by gas chromatograph-combustion-isotope ratio mass spectrometry (GC-C-IRMS) with a Finnigan MAT 252 mass spectrometer interfaced with a Varian 3400 capillary GC. Hydrocarbons were separated by a Poraplot column (25m x 0.32mm ID) with the following temperature program: initial 60°C hold 1 min, increase to 190°C at 5°C/min, hold 5 min. To separate CO2 from CH4 the program was started at 10°C (hold 2 min) increased to 190°C at 5°C/min (hold 5 min). Total error incorporating both accuracy and reproducibility was ±0.5‰ with respect to V-PDB standard. The detection limit for δ13C analysis for this analytical setup is a m/z 44 signal size of 0.3 V.

The δ1H analysis was performed on a continuous flow compound specific hydrogen isotope mass spectrometer that consists of an HP 6890 gas chromatograph (GC) interfaced with a micropyrolysis furnace (1465°C) in line with a Finnigan MAT Delta+XL isotope ratio mass spectrometer. The hydrocarbon gases were separated on a Poraplot Q column (25 m x 0.32 mm ID) with a helium carrier at 2.2 ml/min and the following temperature program: initial 35°C hold 3 min, increase to 180°C at 15°C/min. Total error incorporating both accuracy and reproducibility was ±5‰ with respect to V-SMOW. The detection limit for δ13C analysis for this analytical setup is a m/z 2 signal size of 2 V.

The hydrogen and oxygen isotopic analyses of waters were performed at the Environmental Isotope Laboratory, University of Waterloo, Waterloo, Canada. δ2H2O was determined by manganese reduction at 900°C using a method modified from Coleman et al. (1982). δ18O/H2O analyses were performed by the CO2 equilibration method of Epstein and Mayeda (1953) and Fritz et al. (1986). Reproducibility on duplicate analyses are ±0.4‰ and ±0.1‰ with respect to V-SMOW/ SLAP, for δ2H and δ18O respectively.

For the PCR-amplification of archaeal-domain small subunit ribosomal RNA genes (SSU rDNAs), total DNA was extracted from filters collected from packered boreholes as previously described (Moser et al., 2002), using the Ultra-Clean Soil kit (MoBio, CA) according to the manufacturer’s protocol at Princeton and Portland State Universities.
and Cantor (1969) using the PHYLIP program (Felsenstein, 1993). The joining analysis was calculated according to the algorithm of Jukes from GenBank and the RDP database II using ARB software (Strunk, 2002). The sequences were compiled and aligned with sequences obtained with HaeIII, HhaI, and RsaI restriction enzymes for restriction fragment analysis. In samples where the primer failed to yield a detectable product, nested amplifications were performed using the archaean-specific primers, S-AARCH-0344-a-S-20 (5' - AGG GCG CCC AGC AGG GCG CA-3') (Raskin et al., 1994) and 95R (S - TCC GGC GGT GAM TCC AAT T-3') (DeLong, 1991). PCR was performed for 30 cycles: denaturation for 30 s at 94°C, annealing for 45 s at 54°C, and elongation for 120 s at 72°C. After verification by agarose gel electrophoresis, products were purified and cloned into pCR2.1 using the TOPO TA cloning kit (Invitrogen, CA) following the manufacturer's protocol. At least 40 cloned colonies were picked and reamplified with M13R and T7 primers, and PCR products were subsequently digested with HaeIII, Hhal, and Rsal restriction enzymes for restriction fragment length polymorphism (RFLP) analyses. Clones with distinctive RFLP types were chosen for complete sequencing. Three sequencings were applied to the most dominant RFLP type. Clones represented by each unique RFLP type were assumed to contain the same sequence. The sequences were compiled and aligned with sequences obtained from GenBank and the RDP database II using ARB software (Strunk and Ludwig, 1995; Maitak et al., 2001). A distance matrix for Neighbor Joining analysis was calculated according to the algorithm of Jukes and Cantor (1969) using the PHYLIP program (Felsenstein, 1993). The parsimony and Maximum Likelihood trees were constructed by using the parsimony subprogram within ARB and FASTDNAML (Felsenstein, 1993), respectively. Bootstrap values were calculated on the basis of 1000 iterations. Neighbor Joining, parsimony, Maximum Likelihood, and bootstrap analyses were performed on at least 580 homologous bases. All three produced the same branch pattern.

### 4. RESULTS AND DISCUSSION

The gases from the Karoo Formation at Middelbult mine are coalbed gases consisting primarily of CH4 with smaller amounts of N2 (Table 1). While most samples had O2 concentrations <1%, one sample (MB W135704–2) had an O2 concentration of 6.07% (and a N2/O2 ratio close to 4), reflecting a higher degree of air in this sample and a concomitant decrease in the CH4 concentration to 73.1% due to dilution with air. If the assumption is made that all O2 is due to air contamination, compositional results can be “corrected” by subtracting the amount of O2 and a corresponding amount of N2, Ar, CO2, and renormalizing the corrected volume % values for all the other gases. If the sample MB W135704–2 is corrected in this way, its composition becomes very similar to the 4 others from this site. Such a correction is not routinely applied to samples in this study; however, since all O2 can not be attributed to contamination during sampling. High O2 concentrations (i.e., ~20%) were occasionally observed even at boreholes with relatively high gas flow rates (>0.9 L/min) that would make significant air contamination during sampling unlikely. This phenomenon suggests that at some boreholes the groundwater is already saturated with respect to air before sampling. Atmospheric air has a N2/O2 ratio of 4, therefore, for samples with N2/O2 close to 4, both O2 and N2 can be assumed to be primarily atmospheric in origin, either due to air contamination during sampling or due to air entrainment in groundwater (Table 1).

For all the other samples from Middelbult mine, CH4 con-
ethyl, propane or butane). Small amounts of ethane, propane, concentrations range from 82.9 to 94.5%, with no detectable C₂ due to adsorption or other processes, so such theoretical calculations are not standard practice (Hunt, 1996).

For samples from both the Witwatersrand and Ventersdorp Supergroup formations, CH₄ concentrations were typically between 50 to 90%, except in samples for which O₂ concentrations were particularly high due to contamination during sampling or to air entrainment in groundwater (Table 1). In all cases the two other major components in the gases are N₂ and He.

Figure 3 shows typical ranges of stable isotope values for methane produced by microbial and by thermogenic processes based on empirical data (Whiticar, 1999). Carbon and hydrogen isotope values for CH₄ are a well-established means of distinguishing between methane produced by thermogenic versus microbial processes, providing the isotopic values have not suffered secondary isotope exchange or fractionation (Schoell, 1980; Schoell, 1988). They can also be used to distinguish between microbial CH₄ production by acetate fermentation (AF) versus CO₂ reduction (CR) (Whiticar et al., 1986). The majority of the samples from Merriespruit, Masimong, Beatrix, and Evander mines (Witwatersrand Supergroup) fall within the range of microbiologically produced CH₄ or microbial mixing with a possible thermogenic component (Table 2 and Fig. 3). For Beatrix and Evander, in particular, samples fall along trends that suggest mixing between a ¹³C-depleted microbial CH₄ component and a more ¹³C-enriched and ²H-depleted end member. In contrast, samples from the Ventersdorp Supergroup

![Graph](image)

Fig. 3. δ¹³C(CH₄) and δ²H(CH₄) values for all samples compared to empirically determined fields for thermogenic gas and microbial gas produced by CO₂ reduction (CR) and acetate fermentation (AF) after Whiticar (1999). Samples from the Karoo formation (Middelbult mine) are indicated by (+). All samples from the Witwatersrand Supergroup are open symbols, and all samples from the Ventersdorp Supergroup are solid symbols. Symbols from individual mines are as follows: Merriespruit and Masimong (circles); Beatrix (squares); Evander (triangles); Kloof (diamonds). Kidd Creek data (X) are for abiogenic methane from Sherwood Lollar et al. (2002b). Error bars are ±0.5% for δ¹³C(CH₄) and ±5% for δ²H(CH₄) and are smaller than the plotted symbols. Arrows indicate that samples from Beatrix and Evander fall along apparent mixing trends between a microbial CH₄ component and a more ¹³C-enriched and ²H-depleted end member.

Table 2. Carbon and hydrogen isotopic values (in ‰).

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<th>Mine</th>
<th>Formation</th>
<th>Borehole</th>
<th>δ¹³C(CH₄)</th>
<th>δ²H(CH₄)</th>
<th>δ¹³C(C₂H₆)</th>
<th>δ²H(C₂H₆)</th>
<th>δ¹³C(C₃H₈)</th>
<th>δ²H(C₃H₈)</th>
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<d.l. > below detection limit for isotope analysis.
microbial hydrocarbon gas has a $\delta^{13}C_{\text{CH}_4}$ value more depleted than $-45\%_{\text{o}}$, with $\text{CH}_4/\text{C}_2+$ ratios $\geq 10^5$ (Hunt, 1996). As in Figure 3, the samples from Merriespruit and Masimong (MM5 and MP1) are most consistent with a microbial gas end member, with $\delta^{13}C$ values of $-60.7$ and $-53.7\%_{\text{o}}$, respectively, and CH4/C2+ ratios $>1000$. Due to the fact that C2+ concentrations for EV219hole5 are < detection limit, no CH4/C2+ ratio can be calculated for this sample. The low C2+ concentrations are likely due to air contamination in this sample (N2/O2 ratio = 4.2) causing a decrease in the observed concentration of CH4 and dilution of C2+ peaks to < detection limit. This dilution does not affect $\delta^{13}C$ values however, so the $\delta^{13}C_{\text{CH}_4}$ value for EV219hole5 ($-61.2\%_{\text{o}}$) is shown in Figure 4 in brackets. The similarity in $\delta^{13}C$ and $\delta^2H$ values between MM5 and EV219hole5 supports a predominantly microbial origin via CO2 reduction for both these samples (Figs. 3 and 4). In support of this conclusion, EV219hole5 produced SSU rDNA clones related to the autotrophic Methanosarcina spp. (M. mazei, M. barkeri; (Rouviere et al., 1992; Joulian et al., 1998)), as well as Methanobacterium spp. (autotrophic; (Whittman et al., 1999)) (Fig. 5). Methanobacterium (M. subterraneum; (Kotelnikova et al., 1998)) has been previously cultivated from the deep subsurface. rDNA clones related to Methanosaeta thermoacetophilica and M. concilii (exclusively acetotrophic; (Kamagata et al., 1992)), were also obtained from E219 (Fig. 5). No sample was available from MM5 or MP1, but a sample from MP149 from the same fracture zone as MP1 also produced SSU rDNA clones related to methanogens (Fig. 5). The majority of the data, then, from Beatrix and Evander are consistent with mixing of a microbial end member such as those represented by MM5, MP1, and EV219hole5, with more...
$^{13}$C-enriched gases with higher $C_2^+/C_2^-$ concentrations (lower $CH_4/C_2$ ratios). Although a variety of possible mixing lines can be drawn on Figure 4, the mixing lines depicted bracket most of the samples and can provide an estimate of the amount of microbial $CH_4$ in each sample. Based on a microbial end member similar to MP1, and assuming mixing with a $^{13}$C-enriched end member similar to KL443HWDN, the Beatrix samples are all $>90\%$ microbial in origin, and EV552HW1D is approximately $68\%$ microbial. The most $^{13}$C-depleted Kloof sample (KL441XC56HWDS Hole2) is $18\%$ microbial, based on this calculation. Based on calculating between a microbial end member similar to MM5 and the most $^{13}$C-enriched sample at Evander (EV818NEPD1), the Evander samples range from 5% microbial $CH_4$ (EV818NEPD) to $80\%$ microbial $CH_4$ for EV219ED2 Hole1. These estimates are insensitive to whether the $CH_4/C_2$ ratios for the microbial end members are close to $10^3$, or even higher. They are, however, dependent on the selected $\delta^{13}C$ values for the two end members, and hence provide only a conservative estimate of the relative importance of the microbial component in these samples. In particular, the two mixing lines depicted in Figure 4 assume that the most $^{13}$C-enriched end member observed at Evander (EV818NEPD1) and at Kloof (KL443HWDN) are entirely free of a microbial contribution. If these samples are themselves the product of mixing with microbial $CH_4$ with an even more $^{13}$C-enriched end member, then estimates of the $\%$ microbial contribution would be even larger than those provided above.

Several lines of evidence in fact suggest that the most $^{13}$C-enriched samples are not pure end members but do already contain some contribution from microbial $CH_4$. The molecular and microbiological data indicate the presence of methanogenic archaea in at least some of the boreholes from Merriespruit, Beatrix, and Evander Mines (Fig. 5). In addition, enrichment cultures have identified active methanogenic populations at Merriespruit (Bonin and Boone, 2004). Further, rDNAs related to those of methanogenic archaea (Methanosaeta, Methanothrix, Methanosarcina, Methanobacterium) indicate the presence of methanogens in several Evander samples, including the most $^{13}$C-enriched sample EV818NEPD1 (Fig. 5). Hence, the microbiological evidence supports the idea that this sample is not a pure end member but already is the product of some degree of mixing with microbial hydrocarbons. In contrast, rDNA amplifications for the Kloof samples did not yield any evidence of archaea, much less methanogens. This may, however, be due to the very low biomass in these samples, so a small microbial contribution to the Kloof samples cannot be entirely ruled out. The same fissure sampled by KL441XC56HWDS Hole2 was previously sampled in 1998 from an adjacent borehole (Takai et al., 2001). In the earlier sampling, archaea were detectable using the same DNA extraction and amplification techniques; however, only two lineages, most closely related to Pyrococcus abyssi (non-methanogenic), appeared in the clone library (Takai et al., 2001). Thus, the Venterdorp Supergroup horizons from this mine, while apparently not sterile, support exceedingly weak methanogen populations. The Kloof samples are the deepest (3.3–3.4 kmbls) and hottest (ca. 56–59$^\circ C$) of this sample set. They are also extremely low in biomass (below detection by flow cytometry, data not shown). Witwatersrand Supergroup rocks are present beneath Kloof Mine 4 Shaft, but very deep in unmined horizons below the Ventersdorp Supergroup. If methanogenic populations were to inhabit these rocks, they would have to be hyperthermophilic.

4.2. Origin of the Non-microbial $^{13}$C-Enriched End Member

Earlier theories regarding the origin of hydrocarbon gases in the Witwatersrand basin suggested production of thermogenic gas by thermal decomposition of high molecular weight precursors in the shale layers and carbon seams of the Witwatersrand Supergroup (Cook, 1998), migration of thermogenic hydrocarbons into the Witwatersrand basin from outside (Spangenberg and Frimmel, 2001), or production of hydrocarbons from oil in fluid inclusions (Dutkiewicz et al., 1998). Such a thermogenic origin is plausible for the $^{13}$C-enriched non-microbial end member in this study, given that the isotopic composition of thermogenic $CH_4$ produced from Archean organic matter is likely to be relatively depleted in $^{13}$C compared to Paleozoic and Mesozoic thermogenic gas (Boreham et al., 2001) due to the relative depletion in $^{13}$C of Precambrian organic matter (Schoell and Fellner, 1981). For the samples from EV522 and EV818 in particular, both $\delta^{13}C$ and $\delta^2H$ values (Fig. 3) and $CH_4/C_2$+ ratios (Table 1) are consistent with a thermogenic origin. Hence, the trends from more $^{13}$C-depleted and $^2H$-enriched microbial end members to more $^{13}$C-enriched and $^2H$-depleted samples at Beatrix and Evander (Fig. 3) could be attributed to mixing between microbial $CH_4$ and thermogenic hydrocarbons. Certainly the trends are not consistent with maturity trends which would produce a progressive isotopic enrichment in both $^{13}$C and $^2H$ with increasing maturity. In contrast, the $\delta^2H$ values for the most $^{13}$C-enriched samples from Kloof mine (KL443HWDN1 and KL443HWDN) are considerably more depleted in $^2H$ than typical thermogenic gases (Fig. 3). A significant depletion in $\delta^2H$ values compared to typical thermogenic gases has long been noted for $CH_4$ found in mines at Precambrian Shield sites worldwide and has been suggested to be a characteristic feature of abiogenic $CH_4$ produced by water-rock alteration reactions in this geologic environment (Sherwood Lollar et al., 1993b; Sherwood Lollar et al., 2002b).

4.3. Possible Abiogenic Origin for $^{13}$C-Enriched End Member at Kloof

Analysis of hydrocarbon gases dissolved in saline groundwaters from Kidd Creek mine, Canada, suggested that gases in these Precambrian (2700 Ma) rocks were abiogenic in origin based on a significant depletion in $\delta^2H$ values compared to thermogenic gases and a characteristic $^{13}$C-depletion and $^2H$-enrichment between $CH_4$ and ethane (Fig. 6), consistent with production of higher molecular weight hydrocarbons by polymerization of $CH_4$ (Sherwood Lollar et al., 2002b). This pattern results from kinetically controlled synthesis of higher molecular weight hydrocarbons from lower ones, owing to the fact that $^{12}CH_4$ reacts faster than $^{13}CH_4$ to form chains, so that $^{13}C$ is more likely to be incorporated into larger hydrocarbon chains; whereas the light ($^1H$) isotope will be preferentially eliminated in polymerization reactions owing to preferential cleavage of the lighter $^{12}C^1H$ bond versus the $^{13}C^2H$ bond (Sherwood
Lollar et al., 2002b). In marked contrast, the typical pattern for thermogenic gas consists of a positive correlation of $\delta^{13}$C and $\delta^2$H values between CH$_4$, ethane, and the higher hydrocarbons (propane and butane) (Fig. 6). This pattern of increasing isotopic enrichment in both $^{13}$C and $^2$H with increasing molecular weight for the C$_x$C$_y$ homologues for thermogenic gases results from production of hydrocarbons by thermal cracking of a high molecular weight organic precursor (Des Marais et al., 1994)). Error bars are $\pm 0.5^\circ$ for $\delta^{13}$C, $\delta^2$H values between CH$_4$ and are smaller than the plotted symbols.

While the samples from EV522 and EV818 show an isotopic enrichment in $^{13}$C and $^2$H between CH$_4$ and ethane consistent with a thermogenic origin (Table 2), the most $^{13}$C-enriched Kloof samples (KL443HWND1 and KL443HWDN) show a pattern in Figure 6 more consistent with the proposed abiogenic pattern first described at Kidd Creek Sample (Sherwood Lollar et al., 2002b). While an abiogenic origin has been suggested for a number of hydrocarbon occurrences worldwide (Gold, 1979; Abrajano et al., 1990; Charlo and Donval, 1993; Jenden et al., 1993; Kelley et al., 2001), Sherwood Lollar et al. (2002b) was the first to propose that the pattern of $^{13}$C depletion and $^2$H enrichment between CH$_4$ and ethane could be used to identify abiogenic synthesis of higher hydrocarbons via polymerization from a CH$_4$ precursor. Subsequently abiogenic hydrocarbon gases similar to those first identified at Kidd Creek have been described at four other Precambrian Shield sites in Canada and South Africa (Sherwood Lollar et al., 2002a). Potential mechanisms for abiogenic gas synthesis include: surface-catalyzed polymerization from reduction of CO in the Fischer-Tropsch synthesis (Anderson, 1984); heating or metamorphism of graphite-carbonate-bearing rocks (Giardini and Salotti, 1968; Holloway, 1984); or other vapor-water-rock alteration reactions in the presence of catalytically active metals (McCollom and Seewald, 2001). Confirmation of the exact mechanism responsible for the gases in the Ventsdorp lavas is not possible at this point, but with the exception of serpentinization any of the above processes are feasible in this geologic environment.

4.4. Mixing Constraints Based on $\delta^{18}$O and $\delta^2$H Values

Whether the Witwatersrand and Ventsdorp formations are host rocks (gases formed in situ) or reservoir rocks (where gases are trapped and stored) is an open question. Certainly the abundance of semivertical cross-cutting dykes and fractures throughout the Witwatersrand basin suggest the potential for gas migration and transport along these structures (Fig. 2). $\delta^{18}$O and $\delta^2$H values measured for borehole waters from the sites in this study (Fig. 7) also support mixing. In an earlier study of fissure waters from the mines of the Witwatersrand basin, Lippmann et al. (2003) measured $\delta^{18}$O and $\delta^2$H values that largely fell along the meteoric waterline. Both $^{36}$Cl ages and residence times based on nucleogenic noble gases indicated that these waters were geologically old (1.5–129 Ma) rather than recent meteoric waters, and supported long distance vertical migration along water-bearing fractures, faults, and dykes (Lippmann et al., 2003). While Lippmann et al. (2003) also identified two samples of more highly saline groundwater with $\delta^{18}$O and $\delta^2$H values that fell above the meteoric waterline (21–168 Ma in age based on nucleogenic noble gas residence times), the present paper is the first to demonstrate that fissure waters in the Witwatersrand basin are, in fact, controlled by mixing between two distinct end members (Fig. 7). Fissure waters from the Witwatersrand formation at Beatrix, Merriespruit, and Masimong largely fall on the global meteoric waterline (GMWL). Clearly, the Witwatersrand Supergroup hydrocarbon gas samples with the largest microbial component are associated with groundwaters of paleo-meteoric origin. In contrast, samples from Kloof and Evander range from the GMWL to samples falling significantly above the meteoric waterline, consistent with the effects of water-rock interaction such as alteration of feldspar to clay under low water to rock ratios (Onstott et al., 1997), and consistent with $\delta^{18}$O and $\delta^2$H values found for saline groundwaters from other Precambrian Shield sites worldwide (Frape and Fritz, 1987). As was seen for
the hydrocarbon gas isotopic signatures, the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values for the Klooif and Evander samples are consistent with mixing, in this case between the paleo-meteoric, moderately saline waters with temperatures of 30 to 40°C, and isotopically enriched end members that fall above the meteoric waterline associated with hotter (45–59°C), more saline groundwaters. Significantly, the most saline and isotopically enriched Evander fissure water is also the sample that contains the most $^{13}\text{C}$-enriched CH$_4$ (EV818NEPD1; Figs. 4 and 7). Likewise, the samples with the largest microbial CH$_4$ component (EV219hole5, EV219ED2hole1, and EV552HWD1) lie closest to the meteoric waterline (Fig. 7), while samples EV522CTShole1 and EV522CTShole2 lie in-between these two extremes, just as they do on the mixing lines in Figure 4. Klooif mine samples show a similar distribution with the most $^{18}\text{O}$- and $^2\text{H}$-enriched isotopic groundwaters corresponding to the most $^{13}\text{C}$-enriched CH$_4$ sample–KL443HWD. In contrast, the sample with the most significant microbial component (KL441 HWDShole2) has $^{18}\text{O}$ and $^2\text{H}$ values falling on the meteoric waterline.

5. SUMMARY AND IMPLICATIONS FOR THE DEEP BIOSPHERE

Compositions and $\delta^{13}\text{C}$ and $\delta^2\text{H}$ isotopic values for hydrocarbon gases from mines in the Witwatersrand basin, South Africa, support the widespread occurrence of microbiologically produced hydrocarbon gas in millions of years-old fissure waters. The presence of microbial hydrocarbons is, to a large extent, controlled by the geologic formations in which the gases are found. Samples from the Witwatersrand Supergroup have the largest component of microbial gas, while samples from the Venterdsorp Supergroup support only a smaller component of microbial CH$_4$ (KL441XC56HWDShole2). At all sites, the distribution of $\delta^{13}\text{C}$ values and CH$_4$/C$_2$+ ratios are consistent with mixing between microbial CH$_4$, and a more $^{13}\text{C}$-enriched and C$_2$+ -rich end member. While samples from Evander mine are not inconsistent with a thermogenic origin for the $^{13}\text{C}$-enriched end member, samples from Klooif mine require an additional hydrocarbon source similar in $^{13}\text{C}$ and $^2\text{H}$ isotopic patterns to gases found at other Precambrian Shield sites (Sherwood Lollar et al., 2002a; Sherwood Lollar et al., 2002b). Although a microbial component cannot be ruled out for the Karoo coalbed gases from Middelbult mine, the $\delta^{13}\text{C}$ values and high CH$_4$ content with relatively small amounts of N$_2$ are consistent with production of gas from relatively immature organic source matter (Hunt, 1996) and do not require invoking a microbial contribution.

Mixing trends identified based on hydrocarbon gas compositions and isotopic values are confirmed by $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values measured for fissure waters from the same boreholes. Paleowaters of meteoric origin with temperatures of 30 to 40°C are associated with samples with the largest component of microbial hydrocarbon gas. Samples from the Witwatersrand Supergroup from Beatrix, Merriespruit, and Masimong mines contain the largest component of this paleo-meteoric, microbial end member. In contrast, samples from Evander and Klooif mines reflect mixing between samples with microbial hydrocarbon gases and meteoric $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, and a component of more $^{13}\text{C}$-enriched hydrocarbon gases of non-microbial origin dissolved in the more saline, $^{18}\text{O}$- and $^2\text{H}$-enriched, hotter (45–59°C) fissure waters.

While some mixing of the fissure waters with a small component of modern meteoric water introduced due to mining operations cannot be excluded, the 100 Ma ages established by Lippmann et al. (2003) for these fissure waters suggests that both the Witwatersrand and Venterdsorp end members are geologically old groundwaters stored over long periods in hydrogeologically isolated fracture-controlled flow systems. The case for the existence of indigenous microbial communities in both the Witwatersrand (Takai et al., 2001) and Venterdsorp Supergroup (Takai et al., 2001; Baker et al., 2003; Moser et al., 2003b) is growing. Microbial communities in flowing boreholes, in several cases, have been shown to contain no common SSU rDNA clones in comparisons of libraries from both fissure waters and known sources of contamination (mine service water and air (Baker et al., 2003; Moser et al., 2003b; Onstott et al., 2004). The apparently great age and hydrologic isolation (Takai et al., 2001; Lippmann et al., 2003; Moser et al., 2003b) of the Venterdsorp Supergroup rocks may be consistent with the report of molecular evidence for possible hyperthermophilic organisms related to Pyrococcus abyssi (Takai et al., 2001) in hot waters (50–60°C) from the Venterdsorp Supergroup. The substantial component of microbial hydrocarbons identified in samples from the Witwatersrand Supergroup in this study, and the determination of SSU rDNA sequences related to methanogens from some of these same boreholes, support the widespread occurrence of methanogenic microbial communities in the deep subsurface of the Witwatersrand basin. Stable isotope and gas geochemistry can provide a powerful approach for documenting the presence of such communities in the subsurface.

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